Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	156803	yokomizo.in. or fukuchi.in. or osakada.in. or takagi.in. or ohta. in. or matsumoto.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:04
S2	536711	polyester	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:04
S3	26628	promoter and terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:04
S6	441	3-hydroxyalkan$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:04
S7	18502	hansenula or kluyveromyces or phaffia or pichia or schizosaccharomyces or schwanniomyces or trichospor$ or yarrowia	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:06
S8	132	S3 and (alk3 or xpr2 or alk1)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:16
S9	1	S8 and S1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:16
S10	76	S8 and S2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:16
S11	1	S10 and S6	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:17
S12	74	S10 and S7	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:17
S13	1351	polyhydroxyalkanoate	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:17

S14	196	S13 with gene	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:17
S15	0	S14 and S12	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:18
S16	0	S10 and S14	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:18
S17	132	S14 and S2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 18:18
S18	19	S17 and S7	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:04
S19	261	aeromonas near3 caviae	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:05
S20	89	S13 and S19	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:05
S21	81	S20 and S2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:05
S22	11	S21 and S7	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:08
S23	8	S22 and yeast	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:29
S24	0	alkane-inducible near4 promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:30
S25	5	alkane near2 inducible	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:33

S26	1	S25 and polyester	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:30
S27	800	candida near2 maltosa	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:33
S28	26839	S27 S3	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:33
S29	589	S27 and S3	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:33
S30	85	S29 and S2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/09 19:34
S31	2	S30 and S6	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 12:02
S32	5	"5981257"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 12:03
S33	3	S32 and yeast	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 12:04
S34	0	S33 and pichia	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 12:03
S35	0	S33 and hansenula	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 12:03

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	16518	(|435/254.11|435/254.2|435/70.1|435/483|435/320|536/23.1|536/23|530/402|.ccls.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L2	156803	yokomizo.in. or fukuchi.in. or osakada.in. or takagi.in. or ohta.in. or matsumoto.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L3	37	l1 and L2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L4	536711	polyester	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L5	596	l1 and L4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L6	441	3-hydroxyalkan$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L7	9	l5 and L6	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L8	18502	hansenula or kluyveromyces or phaffia or pichia or schizosaccharomyces or schwanniomyces or trichospor$ or yarrowia	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L9	35	l6 and L8	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L10	1	l9 and l2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:19
L11	26628	promoter and terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:20

L12	59	l6 and L11	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:20
L13	1	l12 and (alk3 or xpr2 or alk1)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/10 14:20

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 FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 14:25:28 ON 10 AUG 2005
L1          3711 S HANSENULA
L2          5198 S KLUYVEROMYCES
L3          9586 S PICHIA
L4           464 S SCHWANNIOMYCES
L5          3764 S TRICHOSPORON
L6        111740 S CANDIDA
L7          1991 S YARROWIA
L8         36094 S "RECOMBINANT PROTEIN EXPRESSION" OR "RECOMBINANT PROTEIN"
L9         42538 S POLYESTER OR HYDROXYALKAN? OR HYDROXYBUT? OR HYDROXYHEXAN?
L10        87481 S ENZYM? (S) SYNTHESIS
L11         1816 S POLYHYDROXYALKAN?
L12       178571 S YOKOMIZO?/AU OR FUKUCHI?/AU OR OSAKADA?/AU OR LEAF?/AU OR OHT
L13            9 S POLYESTER (3W) SYNTHASE
L14            5 DUP REM L13 (4 DUPLICATES REMOVED)
L15       129583 S L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7
L16          872 S L15 AND L12
L17            2 S L16 AND L9
L18            2 DUP REM L17 (0 DUPLICATES REMOVED)
L19          134 S (ALK3 OR XPR2 OR ALK1) (5W) (GENE OR PROMOTER OR TERMINATOR)
L20            3 S L19 AND L12
L21            3 DUP REM L20 (0 DUPLICATES REMOVED)
L22            0 S L19 AND L9
L23           54 S L19 AND L15
L24            6 S L23 AND L8
L25            2 DUP REM L24 (4 DUPLICATES REMOVED)
L26        14256 S AEROMONAS
L27           88 S L26 AND L11
L28            0 S L27 AND L15
L29           46 S L27 AND L12
L30            0 S L29 AND L19
L31            6 S L29 AND POLYESTER
L32            4 DUP REM L31 (2 DUPLICATES REMOVED)

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L14 ANSWER 1 OF 5 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003524469 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12954080
TITLE: Polyester synthases: natural catalysts for plastics.
AUTHOR: Rehm Bernd H A
CORPORATE SOURCE: Institut fur Molekulare Mikrobiologie und Biotechnologie der Westfalischen Wilhelms-Universitat Munster, Corrensstrasse 3, 48149 Munster, Germany.. rehm@uni-muenster.de
SOURCE: Biochemical journal, (2003 Nov 15) 376 (Pt 1) 15-33. Ref: 156
Journal code: 2984726R. ISSN: 1470-8728.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200312
ENTRY DATE: Entered STN: 20031107
Last Updated on STN: 20031219
Entered Medline: 20031209

AB Polyhydroxyalkanoates (PHAs) are biopolyesters composed of hydroxy fatty acids, which represent a complex class of storage polyesters. They are synthesized by a wide range of different Gram-positive and Gram-negative bacteria, as well as by some Archaea, and are deposited as insoluble cytoplasmic inclusions. Polyester synthases are the key enzymes of polyester biosynthesis and catalyse the conversion of (R)-hydroxyacyl-CoA thioesters to polyesters with the concomitant release of CoA. These soluble enzymes turn into amphipathic enzymes upon covalent catalysis of polyester-chain formation. A self-assembly process is initiated resulting in the formation of insoluble cytoplasmic inclusions with a phospholipid monolayer and covalently attached polyester synthases at the surface. Surface-attached polyester synthases show a marked increase in enzyme activity. These polyester synthases have only recently been biochemically characterized. An overview of these recent findings is provided. At present, 59 **polyester synthase** structural genes from 45 different bacteria have been cloned and the nucleotide sequences have been obtained. The multiple alignment of the primary structures of these polyester synthases show an overall identity of 8-96% with only eight strictly conserved amino acid residues. Polyester synthases can been assigned to four classes based on their substrate specificity and subunit composition. The current knowledge on the organization of the **polyester synthase** genes, and other genes encoding proteins related to PHA metabolism, is compiled. In addition, the primary structures of the 59 PHA synthases are aligned and analysed with respect to highly conserved amino acids, and biochemical features of polyester synthases are described. The proposed catalytic mechanism based on similarities to alpha/beta-hydrolases and mutational analysis is discussed. Different threading algorithms suggest that polyester synthases belong to the alpha/beta-hydrolase superfamily, with a conserved cysteine residue as catalytic nucleophile. This review provides a survey of the known biochemical features of these unique enzymes and their proposed catalytic mechanism.

L14 ANSWER 2 OF 5 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:348122 BIOSIS
DOCUMENT NUMBER: PREV200200348122
TITLE: **Polyester synthase** and a gene coding for the same.
AUTHOR(S): Doi, Yoshiharu [Inventor, Reprint author]; Fukui, Toshiaki [Inventor]; Matsusaki, Hiromi [Inventor]
CORPORATE SOURCE: Saitama, Japan
ASSIGNEE: Japan Science and Technology Corporation, Saitama, Japan; The Institute of Physical and Chemical Research, Saitama, Japan
PATENT INFORMATION: US 6391611 20020521

SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (May 21, 2002) Vol. 1258, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Jun 2002
Last Updated on STN: 19 Jun 2002

AB The present invention relates to a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a sequence of SEQ ID NO:1 where in one or more amino acids are deleted, replaced or added, and the polypeptide having **polyester synthase** activity; a **polyester synthase** gene comprising DNA coding for the above polypeptide; a recombinant vector comprising the gene; and a transformant transformed with the recombinant vector is also provided.

L14 ANSWER 3 OF 5 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002299017 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12038975
TITLE: In vitro evolution of a polyhydroxybutyrate synthase by intragenic suppression-type mutagenesis.
AUTHOR: Taguchi Seiichi; Nakamura Hirofumi; Hiraishi Tomohiro; Yamato Ichiro; Doi Yoshiharu
CORPORATE SOURCE: Polymer Chemistry Laboratory, RIKEN Institute, Hirosawa, Wako, Saitama 351-0198, Japan.. staguchi@isc.meiji.ac.jp
SOURCE: Journal of biochemistry, (2002 Jun) 131 (6) 801-6. Journal code: 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20020602
Last Updated on STN: 20030103
Entered Medline: 20030102

AB In vitro evolution was applied to obtain highly active mutants of Ralstonia eutropha **polyester synthase** (PhbC(Re)), which is a key enzyme catalyzing the formation of polyhydroxybutyrate (PHB) from (R)-3-hydroxybutyryl-CoA (3HB-CoA). To search for beneficial mutations for activity improvement of this enzyme, we have conducted multi-step mutations, including activity loss and intragenic suppression-type activity reversion. Among 259 revertants, triple mutant E11S12 was obtained as the most active one via PCR-mediated secondary mutagenesis from mutant E11 with a single mutation (Ser to Pro at position 80), which exhibited reduced activity (as low as 27% of the wild-type level) but higher thermostability compared to the wild-type enzyme. Mutant E11S12 exhibited up to 79% of the wild-type enzyme activity. Mutation separation of E11S12 revealed that the replacement of Phe by Ser at position 420 (F420S), located in a highly conserved alpha/beta hydrolase fold region, of the E11S12 mutant contributes to the improvement of the enzyme activity. A purified sample of the genetically engineered mutant, termed E11S12-1, with the F420S mutation alone was found to exhibit a 2.4-fold increase in specific activity toward 3HB-CoA, compared to the wild-type.

L14 ANSWER 4 OF 5 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:278982 BIOSIS
DOCUMENT NUMBER: PREV200000278982
TITLE: **Polyester synthase** gene and process for producing polyester.
AUTHOR(S): Fukui, Toshiaki [Inventor, Reprint author]; Doi, Yoshiharu [Inventor]
CORPORATE SOURCE: Saitama, Japan
ASSIGNEE: The Institute of Physical and Chemical Research, Saitama, Japan
PATENT INFORMATION: US 5981257 19991109
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 9, 1999) Vol. 1228, No. 2. e-file.

CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Jul 2000
Last Updated on STN: 7 Jan 2002

AB The present invention relates to a **polyester synthase** gene coding for a polypeptide containing the amino acid sequence of SEQ ID NO:2 or a sequence where in the amino acid sequence of SEQ ID NO:2, one or more amino acids are deleted, replaced or added, the polypeptide bringing about **polyester synthase** activity; a gene expression cassette comprising the **polyester synthase** gene and either of open reading frames located upstream and downstream of the gene; a recombinant vector comprising the gene expression cassette; a transformant transformed with the recombinant vector; and a process for producing polyester by culturing the transformant in a medium and recovering polyester from the resulting culture.

L14 ANSWER 5 OF 5 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:278859 BIOSIS
DOCUMENT NUMBER: PREV200000278859
TITLE: **Polyester synthase** and a gene coding for the same.
AUTHOR(S): Doi, Yoshiharu [Inventor, Reprint author]; Fukui, Toshiaki [Inventor]; Matsusaki, Hiromi [Inventor]
CORPORATE SOURCE: Saitama, Japan
ASSIGNEE: Japan Science and Technology Corporation; The Institute of Physical and Chemical Research
PATENT INFORMATION: US 5968805 19991019
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 19, 1999) Vol. 1227, No. 3. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Jul 2000
Last Updated on STN: 7 Jan 2002

AB The present invention relates to a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a sequence of SEQ ID NO:1 where in, one or more amino acids are deleted, replaced or added, and the polypeptide having **polyester synthase** activity. A **polyester synthase** gene comprising DNA coding for the above polypeptide; a recombinant vector comprising the gene; and a transformant transformed with the recombinant vector is also provided.

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L18 ANSWER 1 OF 2 MEDLINE on STN
ACCESSION NUMBER: 2005367078 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15988790
TITLE: Enzymatic transformation of bacterial polyhydroxyalkanoates into repolymerizable oligomers directed towards chemical recycling.
AUTHOR: Kaihara Sachiko; Osanai Yasushi; Nishikawa Kimihito; Toshima Kazunobu; **Doi Yoshiharu**; Matsumura Shuichi
CORPORATE SOURCE: Faculty of Science and Technology, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan.
SOURCE: Macromol Biosci, (2005 Jul 14) 5 (7) 644-52.
Journal code: 101135941. ISSN: 1616-5187.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20050719
Last Updated on STN: 20050731

AB The enzymatic transformation into an oligomer was carried out with the objective of developing the chemical recycling of bacterial polyesters. Poly(R-3-**hydroxyalkanoate**)s (PHAs), such as poly[(R-3-**hydroxybutyrate**)-co-12%(R-3-**hydroxyhexanoate**)] and poly[(R-3-**hydroxybutyrate**)-co-12%(R-3-hydroxyvalerate)], were degraded by granulated **Candida** antarctica lipase B immobilized on hydrophilic silica (lipase GCA) in a diluted organic solvent at 70 degrees C. The degradation products were cyclic oligomers having a molecular weight of a few hundreds. The obtained cyclic oligomer was readily repolymerized by the same lipase (lipase GCA) to produce the corresponding **polyester** in a concentrated solution. The cyclic oligomer was copolymerized with epsilon-caprolactone using lipase to produce the corresponding terpolymers having an Mw of 21,000. This is the first example of the enzymatic chemical recycling of bacterial PHAs using lipase. Poly(R-3-**hydroxybutyrate**) [P(3HB)] was also degraded into the linear-type R-3HB monomer to trimer by P(3HB)-depolymerase (PHBDP) in phosphate buffer at 37 degrees C. The degradation using PHBDP required a longer reaction time compared with the lipase-catalyzed degradation in organic solvent. The monomer composition of the oligomer depended on the origin of the PHBDP. The R-3HB monomer was predominately produced by PHBDP from Pseudomonas stutzeri, while the R-3HB dimer was produced by PHBDP from Alcaligenes faecalis T1. Repolymerization of these oligomers by lipase in concentrated organic solvent produced a relatively low-molecular-weight P(3HB) (e.g., Mw=2,000). Degradation of P(3HB) by lipase in organic solvent into repolymerizable cyclic oligomer and degradation of P(3HB) by PHBDP in buffer into hydroxy acid type R-3HB dimer.

L18 ANSWER 2 OF 2 EMBASE
on STN
ACCESSION NUMBER: 2003417874 EMBASE
TITLE: Lipase-catalyzed synthesis of polyesters from anhydride derivatives involving dehydration.
AUTHOR: Uyama H.; Wada S.; **Fukui T.**; Kobayashi S.
CORPORATE SOURCE: S. Kobayashi, Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 606-8501, Japan. kobayasi@mat.polym.kyoto-u.ac.jp
SOURCE: Biochemical Engineering Journal, (2003) Vol. 16, No. 2, pp. 145-152.
Refs: 31
ISSN: 1369-703X CODEN: BEJOFV
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20031030
Last Updated on STN: 20031030

AB Lipase-catalyzed synthesis of aliphatic polyesters has been achieved by using anhydride derivatives as starting substrate. **Candida** antarctica lipase efficiently catalyzed the dehydration reaction of polyanhydrides and glycols in toluene, yielding the corresponding polyesters. Effects of the reaction parameters on the polymer yield and molecular weight have been systematically investigated. The dehydration reaction also proceeded in water and supercritical carbon dioxide (scCO(2)). The reaction behaviors depended on the monomer structure and reaction media. Cyclic anhydrides, succinic, glutaric, and diglycolic anhydrides, were enzymatically polymerized with α,ω-alkylene glycols in toluene to give the polyesters. Under appropriate reaction conditions, the molecular weight reached 1 x 10 (4).

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L21 ANSWER 1 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:416257 BIOSIS
DOCUMENT NUMBER: PREV200200416257
TITLE: YlALK1 encoding the cytochrome P450ALK1 in Yarrowia lipolytica is transcriptionally induced by n-alkane through two distinct cis-elements on its promoter.
AUTHOR(S): Sumita, Toru; Iida, Toshiya; Yamagami, Setsu; Horiuchi, Hiroyuki; **Takagi, Masamichi; Ohta, Akinori** [Reprint author]
CORPORATE SOURCE: Department of Biotechnology, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan
aaohta@mail.ecc.u-tokyo.ac.jp
SOURCE: Biochemical and Biophysical Research Communications, (June 28, 2002) Vol. 294, No. 5, pp. 1071-1078. print.
CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Jul 2002
Last Updated on STN: 31 Jul 2002

AB The YlALK1 gene, which encodes cytochrome P450ALK1, plays a primary role in the assimilation of n-decane by yeast Yarrowia lipolytica and is inducible by n-decane at the transcriptional level. Deletion analysis of the YlALK1 promoter revealed that a 95-bp region on the YlALK1 promoter (from the position -400 to -304 upstream of the ATG codon) is essential for the induction by n-decane and we named this region ARR1 (alkane-responsive region). ARR1 was found to be made up of two different elements, ARE1 (alkane-responsive element 1; from -394 to -371) and ARE2 (from -325 to -305). By electrophoretic mobility shift assay, we found that the respective elements gave specific shift bands with the extracts from Y. lipolytica cells grown on n-alkane, but not much evidently from the cells grown on glycerol or glucose. This suggests that proteins that specifically bind to these elements are present and their binding or synthesis is dependent on n-alkane.

L21 ANSWER 2 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:134230 BIOSIS
DOCUMENT NUMBER: PREV199800134230
TITLE: Isozyme function of n-alkane-inducible cytochromes P450 in Candida maltosa revealed by sequential gene disruption.
AUTHOR(S): Ohkuma, Moriya; Zimmer, Thomas; Iida, Tokshiya; Schunck, Wolf-Hagen; **Ohta, Akinori; Takagi, Masamichi** [Reprint author]
CORPORATE SOURCE: Lab. Cellular Genetics, Dep. Biotechnol., Univ. Tokyo, Bunkyo-ku, Tokyo 113, Japan
SOURCE: Journal of Biological Chemistry, (Feb. 13, 1998) Vol. 273, No. 7, pp. 3948-3953. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Mar 1998
Last Updated on STN: 20 Mar 1998

AB An n-alkane-assimilating yeast Candida maltosa contains multiple n-alkane-inducible forms of cytochromes P450 (P450alk), which can be assumed to catalyze terminal hydroxylation of n-alkanes in the assimilation pathway. Eight structurally related P450alk genes have been identified. In the present study, the function of four major isoforms of P450alk (encoded by ALK1, ALK2, ALK3, and ALK5 genes) was investigated by sequential gene disruption. Auxotrophic markers used for the selection of disrupted strains were regenerated repeatedly through either mitotic recombination between heterozygous alleles of the diploid genome or directed deletion of the marker gene, to allow sequential gene disruptions within a single strain. The strain depleted of all four isoforms could not utilize n-alkanes for growth, providing direct evidence that P450alk is essential for n-alkane assimilation. Growth properties of a series of intermediate disrupted strains, plasmid-based complementation, and enzyme assays after heterologous expression of single isoforms revealed (i) that each of the four individual isoforms is alone sufficient to allow growth

on long chain n-alkane; (ii) that the ALK1-encoding isoform is the most versatile and efficient P450alk form, considering both its enzymatic activity and its ability to confer growth on n-alkanes of different chain length; and (iii) that the ALK5-encoding isoform exhibits a rather narrow substrate specificity and thus cannot support the utilization of short chain n-alkanes.

L21 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:338779 BIOSIS
DOCUMENT NUMBER: PREV199699061135
TITLE: Peroxisome proliferators activate cytochrome P450 genes in an alkane-assimilating yeast, Candida maltosa.
AUTHOR(S): Ohtomo, Ryo; Kobayashi, Keisuke; Muraoka, Shin-Ichiro; Ohkuma, Moriya; **Ohta, Akinori; Takagi, Masamichi**
CORPORATE SOURCE: Dep. Biotechnol., Univ. Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan
SOURCE: Biochemical and Biophysical Research Communications, (1996) Vol. 222, No. 3, pp. 790-793.
CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Jul 1996
Last Updated on STN: 26 Sep 1996

AB Candida maltosa can assimilate n-alkane as a sole carbon source and cytochromes P450ALK (P450ALK) are critical for the first oxidation step. Four major P450ALKs that are encoded by genes ALK1, ALK2, ALK3 and ALK5 are induced by n-alkane and repressed by glucose at the transcriptional level. In the present work, we found that all these four genes but ALK5 are transcriptionally activated in response to a peroxisome proliferator, clofibrate. This is the first report on the peroxisome proliferator responsive gene expression in lower eucaryotes.

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L32 ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002265536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11976116
TITLE: Enhanced accumulation and changed monomer composition in **polyhydroxyalkanoate** (PHA) copolyester by in vitro evolution of **Aeromonas** caviae PHA synthase.
AUTHOR: Kichise Tomoyasu; Taguchi Seiichi; **Doi Yoshiharu**
CORPORATE SOURCE: Polymer Chemistry Laboratory, RIKEN Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan.
SOURCE: Applied and environmental microbiology, (2002 May) 68 (5) 2411-9.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020514
Last Updated on STN: 20020713
Entered Medline: 20020712

AB By in vitro evolution experiment, we have first succeeded in acquiring higher active mutants of a synthase that is a key enzyme essential for bacterial synthesis of biodegradable **polyester**, **polyhydroxyalkanoate** (PHA). **Aeromonas** caviae FA440 synthase, termed PhaC(Ac), was chosen as a good target for evolution, since it can synthesize a PHA random copolyester of 3-hydroxybutyrate and 3-hydroxyhexanoate [P(3HB-co-3HHx)] that is a tough and flexible material compared to polyhydroxybutyrate (PHB) homopolyester. The in vitro enzyme evolution system consists of PCR-mediated random mutagenesis targeted to a limited region of the phaC(Ac) gene and screening mutant enzymes with higher activities based on two types of **polyester** accumulation system by using Escherichia coli for the synthesis of PHB (by JM109 strain) (S. Taguchi, A. Maehara, K. Takase, M. Nakahara, H. Nakamura, and Y. Doi, FEMS Microbiol. Lett. 198:65-71, 2001) and of P(3HB-co-3HHx) [by LS5218 [fadR601 atoC(Con)] strain]. The expression vector for the phaC(Ac) gene, together with monomer-supplying enzyme genes, was designed to synthesize PHB homopolyester from glucose and P(3HB-co-3HHx) copolyester from dodecanoate. Two evolved mutant enzymes, termed E2-50 and T3-11, screened through the evolution system exhibited 56 and 21% increases in activity toward 3HB-coenzyme A, respectively, and consequently led to enhanced accumulation (up to 6.5-fold content) of P(3HB-co-3HHx) in the recombinant LS5218 strains. Two single mutations in the mutants, N149S for E2-50 and D171G for T3-11, occurred at positions that are not highly conserved among the PHA synthase family. It should be noted that increases in the 3HHx fraction (up to 16 to 18 mol%) were observed for both mutants compared to the wild type (10 mol%).

L32 ANSWER 2 OF 4 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2001272418 EMBASE
TITLE: Characterization of 13 kDa granule-associated protein in **Aeromonas** caviae and biosynthesis of **polyhydroxyalkanoates** with altered molar composition by recombinant bacteria.
AUTHOR: **Fukui T.**; Kichise T.; Iwata T.; **Doi Y.**
CORPORATE SOURCE: Y. Doi, Polymer Chemistry Laboratory, RIKEN Institute, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan. ydoi@postman.riken.go.jp
SOURCE: Biomacromolecules, (2001) Vol. 2, No. 1, pp. 148-153.
Refs: 34
ISSN: 1525-7797 CODEN: BOMAF6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20010823

Last Updated on STN: 20010823

AB Analysis of native poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) [P(3HB-co-3HHx)] inclusions from **Aeromonas** caviae FA440 revealed that ORF1 (a 348-bp gene located immediately upstream of phaC(Ac)) encodes a 13-kDa granule-associated protein, which was referred to as phaP(Ac). Several recombinant strains of A. caviae were constructed and conducted to analyze their PHA-producing abilities. A transconjugant of FA440 harboring additional copies of phaPCJ(Ac) genes accumulated P(3HB-co-3HHx) copolyesters with much higher 3HHx composition (46-63 mol %) than wild-type strain from alkanoates or olive oil. Deletion analysis revealed that overexpression of phaJ(Ac) encoding monomer-supplying (R)-hydratase was not a reason for the compositional change in the recombinant strains. PHA synthase activity in PHA inclusion fraction from the transconjugant composed of 60 mol % of 3HHx was 10-fold higher than that from the strain FA440 with 13 mol % of 3HHx, suggesting an importance of the level of PHA synthase activity for controlling the PHA composition in vivo.

L32 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:227789 BIOSIS
DOCUMENT NUMBER: PREV200000227789
TITLE: Molecular cloning of two (R)-specific enoyl-CoA hydratase genes from Pseudomonas aeruginosa and their use for **polyhydroxyalkanoate** synthesis.
AUTHOR(S): Tsuge, Takeharu; **Fukui, Toshiaki**; Matsusaki, Hiromi; Taguchi, Seiichi; Kobayashi, Genta; Ishizaki, Ayaaki; **Doi, Yoshiharu** [Reprint author]
CORPORATE SOURCE: Polymer Chemistry Laboratory, RIKEN Institute, 2-1 Hirosawa, Wako-shi, Saitama, 351-0198, Japan
SOURCE: FEMS Microbiology Letters, (March 15, 2000) Vol. 184, No. 2, pp. 193-198. print.
CODEN: FMLED7. ISSN: 0378-1097.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Jun 2000
Last Updated on STN: 5 Jan 2002

AB Two Pseudomonas aeruginosa genes, termed phaJ1Pa and phaJ2Pa, homologous to the **Aeromonas** caviae (R)-specific enoyl-CoA hydratase gene(phaJAc) were cloned using a PCR technique to investigate the monomer-supplying ability for **polyhydroxyalkanoate** (PHA) synthesis from beta-oxidation cycle. Two expression plasmids for phaJ1Pa and phaJ2Pa were constructed and introduced into Escherichia coli DH5alpha strain. The recombinants harboring phaJ1Pa or phaJ2Pa showed high (R)-specific enoyl-CoA hydratase activity with different substrate specificities, that is, specific for short chain-length enoyl-CoA or medium chain-length enoyl-CoA, respectively. In addition, co-expression of these two hydratase genes with PHA synthase gene in E. coli LS5218 resulted in the accumulation of PHA up to 14-29 wt% of cell dry weight from dodecanoate as a sole carbon source. It has been suggested that phaJ1Pa and phaJ2Pa products have the monomer-supplying ability for PHA synthesis from beta-oxidation cycle.

L32 ANSWER 4 OF 4 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 97356372 EMBASE
DOCUMENT NUMBER: 1997356372
TITLE: Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxy-heptanoate) terpolymers by recombinant Alcaligenes eutrophus.
AUTHOR: **Fukui T.**; Kichise T.; Yoshida Y.; **Doi Y.**
CORPORATE SOURCE: T. Fukui, Polymer Chemistry Laboratory, Institute Physical Chemical Research, Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan. ydoi@postman.riken.go.jp
SOURCE: Biotechnology Letters, (1997) Vol. 19, No. 11, pp. 1093-1097.
Refs: 14
ISSN: 0141-5492 CODEN: BILED3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 971204
Last Updated on STN: 971204

AB Recombinant strains of Alcaligenes eutrophus harboring the **polyhydroxyalkanoate** (PHA) synthase gene of **Aeromonas** caviae synthesized poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyheptanoate) terpolymers from alkanoic acids of odd carbon numbers. The results indicated the specificity of PHA synthase of A. caviae toward 3-hydroxyalkanoate units from C4 to C7. The composition of the polyesters formed varied as the carbon numbers of the alkanoic acids fed increased.

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